

**CLAIMS:**

1. A method for detecting the presence or level of alkylated cytosine in a sample of genomic or mitochondrial double stranded DNA from an individual, the method comprising:
  - 5 (a) obtaining a sample of the double stranded DNA from the individual;
  - (b) converting at least one region of the double stranded DNA to single stranded DNA;
  - (c) reacting a target region of the single stranded DNA from step (b) with at least one enzyme, the enzyme differentially modifying alkylated cytosine and cytosine; and
  - 10 (d) determining the level of enzymatic modification of the target region by the enzyme.
2. A method according to claim 1 wherein the single stranded DNA is reacted with the enzyme under conditions such that the enzyme reacts substantially only with either alkylated cytosine or cytosine in the single stranded DNA but not both.
- 15 3. A method according to claim 1 wherein the enzyme is capable of reacting substantially with only one of alkylated cytosine or cytosine in the single stranded DNA.
4. A method according to any one of claims 1 to 3 wherein the conversion of the region of the double stranded DNA to the single stranded DNA comprises at least partially separating the two strands of the double stranded DNA.
- 20 5. A method according to claim 4 wherein one or more strand displacing probes are utilised to at least partially separate the two strands of the double stranded DNA.
6. A method according to claim 5 wherein the or each strand displacing probe is independently selected from the group consisting of nucleic acid analogue probes, PNA containing probes, LNA containing probes, PNA probes and LNA probes.
- 25 7. A method according to claim 4 further comprising inhibiting annealing of the two strands of the double stranded DNA together once they have been separated to facilitate access to the target region by the enzyme.

8. A method according to claim 7 further comprising hybridising at least one probe with a strand of the double stranded DNA following separation of the two strands to thereby inhibit the annealing of the two strands together.

9. A method according to claim 8 wherein the at least one probe is independently selected from the group consisting of sense probes, looping probes, antisense probes and mixtures thereof.

10. A method according to claim 8 wherein at least two said probes are hybridised with the strand of the double stranded DNA, one of the probes hybridising with a region of the strand downstream of the target region and a further of the probes hybridising with a region of the strand upstream of the target region.

11. A method according to claim 8 wherein the probe hybridises with upstream and downstream regions of the strand which flank the target region such that a loop or bubble which incorporates the target region is formed in the strand.

12. A method according to claim 8 wherein the probe hybridises with the strand of the double stranded DNA either side of the target region and the probe has a middle region of non-complementary sequence that does not hybridise with the target region such that a loop or bubble incorporating the target region is formed in the strand.

13. A method according to claim 12 wherein the middle region of the probe incorporates inverted repeats that hybridise together following hybridisation of the probe with the strand of the double stranded DNA.

14. A method according to any one of claims 1 to 13 wherein the determination of the level of enzymatic modification of the single stranded DNA comprises analysing for sequence variations arising from the enzymatic modification of the target region of the single stranded DNA by the enzyme.

25 15. A method according to claim 14 wherein the determination of the level of enzymatic modification comprises subjecting the target region of the single stranded DNA to an amplification process involving thermocycling and primers to obtain an amplified product, and analysing the amplified product for sequence variations.

16. A method according to claim 15 wherein the analysis of the amplified product comprises subjecting the amplified product to a technique selected from the group consisting of nucleic acid sequencing, polymerase chain reaction techniques, restriction enzyme digests and techniques involving the use of probes that bind to specific nucleic acid sequences.
17. A method according to claim 16 wherein the analysis of the amplified product comprises subjecting the amplified product to a polymerase chain reaction technique.
18. A method according to any one of claims 1 to 17 wherein the at least one enzyme deaminates alkylated cytosine or cytosine in the target region of the single stranded DNA.
19. A method according to any one of claims 1 to 18 wherein a combination of different said enzymes are employed to differentially modify alkylated cytosine and cytosine in the target region.
20. A method according to any one of claims 1 to 19 wherein the or each enzyme is independently a deaminase enzyme or a catalytic fragment, variant, homologue, or a modified form or mutant form thereof, having deaminase activity of the enzyme.
21. A method according to claim 20, wherein the enzyme is selected from the group consisting of ApoBRe, AID, and AID mutant R35E/R36D.
22. A method according to any one of claims 1 to 21 comprising detecting the presence or level of alkylated cytosine in a gene or a non-coding region of a gene, or a fragment thereof.
23. A method according to claim 22 comprising detecting the presence or level of alkylated cytosine in a 5' untranslated region of a gene.
24. A method according to claim 23 wherein the level of alkylated cytosine comprises hypermethylation.
25. A method according to claim 23 wherein the level of alkylated cytosine comprises hypomethylation.

26. A method according to claim 23 wherein the gene is selected from the group consisting of *p16*, *E-cadherin*, the VHL gene, *BRCA1*, *p15*, hMLH1, ER, HIC1, MDG1, GST- $\pi$ , O<sup>6</sup>-MGMT, calcitonin, *myo-D*, urokinase and S100A4.

27. A method according to any one of claims 1 to 26 wherein the detection of an altered 5 level of alkylated cytosine in the target region of the single stranded DNA is a marker for a disease or condition.

28. A method according to claim 27 wherein the disease or condition is cancer.

29. A method according to claim 28 wherein the cancer is selected from the group 10 consisting of lung cancer, breast cancer, colon cancer, bladder cancer, liver cancer, head and neck tumours, prostate cancer, renal cell tumours, leukemias, Burkitt lymphomas, brain tumours and carcinoma.

30. A method according to any one of the claims 1 to 23 further comprising diagnosing a disease or condition in the individual on the basis of the presence or the level of alkylated cytosine in the target region of the single stranded DNA.

15 31. A method according to claim 30 wherein the disease or condition comprises a cancer selected from the group consisting of lung cancer, breast cancer, colon cancer, bladder cancer, liver cancer, head and neck tumours, prostate cancer, renal cell tumours, leukemias, Burkitt lymphomas, brain tumours and carcinoma.

32. A method according to any one of claims 1 to 23 wherein the presence or level of 20 the alkylated cytosine is detected to indicate the presence or absence of foetal DNA.

33. A method according to any one of claims 1 to 23 wherein the presence or level of the alkylated cytosine is detected for indicating the presence or absence of an altered gene imprinting state.

25 34. A method according to any one of claims 1 to 33 wherein the presence or level of the alkylated cytosine is detected to indicate the presence or absence of a pathogen or microorganism.

35. A method according to any one of claims 1 to 34 wherein the alkylated cytosine is methylated cytosine.

36. A method according to claim 35 wherein the methylated cytosine is 5-methylcytosine.
37. A method according to any one of claims 1 to 36 wherein the double stranded DNA is genomic DNA.
- 5 38. A kit for use in a method of detecting the presence or level of alkylated cytosine in a sample of genomic or mitochondrial double stranded DNA from an individual as defined in any one of claims 1 to 37, wherein the kit comprises one or more reagents for performing the method and instructions for use.